

WHAT IS CLAIMED IS:

5 1. A modified pore-subunit polypeptide comprising a pore-subunit polypeptide covalently linked to at least a first sensing moiety, wherein said modified pore-subunit polypeptide assembles into an oligomeric pore assembly in the presence of a plurality of pore-subunit polypeptides.

10 2. The modified polypeptide of claim 1, wherein said sensing moiety is a functional group.

15 3. The modified polypeptide of claim 2, wherein said functional group is an analyte-binding functional group.

20 4. The modified polypeptide of claim 2, wherein said functional group is a synthetic molecule.

25 5. The modified polypeptide of claim 4, wherein said functional group is a calixarene or a crown ether.

30 6. The modified polypeptide of claim 2, wherein said functional group is a naturally occurring molecule.

7. The modified polypeptide of claim 6, wherein said functional group is an enzyme inhibitor, a hapten, a nucleotide, an amino acid, a lipid, a toxin, a saccharide, a chelator or a cyclodextrin.

8. The modified polypeptide of claim 1, wherein said sensing moiety is a polymer.

5 9. The modified polypeptide of claim 8, wherein said polymer is polyethylene glycol
(PEG).

10 10. The modified polypeptide of claim 9, wherein said polymer is polyethylene glycol
(PEG)-biotin.

11. The modified polypeptide of claim 8, wherein said polymer is an analyte-binding
polymer.

12. The modified polypeptide of claim 11, wherein said polymer is an oligonucleotide, an
oligosaccharide or a peptide.

13. The modified polypeptide of claim 1, wherein said sensing moiety binds to a metal,
metal ion, a toxin, an enzyme, a nucleotide, an oligonucleotide, an amino acid, a peptide, a
saccharide, a hapten, a lipid or an antibody or antigen-binding fragment thereof.

25 14. The modified polypeptide of claim 1, wherein said sensing moiety responds to a
change in the type or amount of a biological or chemical constituent in the environment of
said oligomeric pore assembly.

30 15. The modified polypeptide of claim 1, wherein said sensing moiety responds to a
change in the physical environment of said oligomeric pore assembly.

16. The modified polypeptide of claim 15, wherein said sensing moiety responds to a change in pH, light, voltage or temperature.

5

17. The modified polypeptide of claim 1, wherein said polypeptide is covalently linked to at least a first and at least a second sensing moiety.

10 18. The modified polypeptide of claim 17, wherein said at least a first sensing moiety is distinct from said at least a second sensing moiety.

15
20
25

19. The modified polypeptide of claim 17, wherein said at least a first sensing moiety is the same as said at least a second sensing moiety.

20. The modified polypeptide of claim 1, wherein said polypeptide is a staphylococcal hemolysin polypeptide, a porin, a complement pore polypeptide, a hemolysin C polypeptide or a streptolysin O polypeptide.

25 21. The modified polypeptide of claim 20, wherein said polypeptide is a staphylococcal alpha hemolysin polypeptide.

22. The modified polypeptide of claim 21, wherein said polypeptide is a mutant staphylococcal alpha hemolysin polypeptide comprising at least a first heterologous amino acid.

30

23. The modified polypeptide of claim 22, wherein said mutant staphylococcal alpha hemolysin polypeptide comprises a cysteine residue in place of serine at position 106 of the

wild-type staphylococcal alpha hemolysin polypeptide or a cysteine residue in place of lysine at position 8 of the wild-type staphylococcal alpha hemolysin polypeptide.

5 24. A modified pore-subunit polypeptide comprising a staphylococcal alpha hemolysin pore-subunit polypeptide covalently linked to at least a first sensing moiety, wherein said modified pore-subunit polypeptide assembles into a heptameric pore assembly in the presence of a plurality of staphylococcal alpha hemolysin pore-subunit polypeptides.

10

15 25. An oligomeric pore assembly comprising a number of pore-subunit polypeptides sufficient to form a pore, wherein at least one of said pore-subunit polypeptides is a modified pore-subunit polypeptide comprising a pore-subunit polypeptide covalently linked to a sensing moiety.

15

20 26. The pore assembly of claim 25, wherein said pore assembly comprises at least a first and second of said modified pore-subunit polypeptides.

20

25 27. The pore assembly of claim 26, wherein said first and second modified pore-subunit polypeptides are each covalently linked to a distinct sensing moiety.

25

28. The pore assembly of claim 26, wherein said pore assembly comprises a plurality of said modified pore-subunit polypeptides.

30

29. The pore assembly of claim 28, wherein said pore assembly is comprised completely of said modified pore-subunit polypeptides.

30. The pore assembly of claim 25, wherein said pore assembly comprises 7 pore-subunit polypeptides.

5 31. A biosensor device comprising the pore assembly of claim 25.

10 32. A method of detecting the presence of an analyte in a sample, comprising contacting said sample with the pore assembly of claim 25, and detecting an electrical current through at least a first channel, wherein a modulation in current compared to a current measurement in a control sample lacking said analyte indicates the presence of said analyte in said sample.

15 33. The method of claim 32, wherein said electrical current is detected through a single channel.

20 34. The method of claim 32, wherein said electrical current is detected through at least two channels.

35. The method of claim 32, wherein said analyte is known.

25 36. The method of claim 32, wherein said analyte is unknown.

30 37. The method of claim 32, wherein said analyte is an oligonucleotide.

38. The method of claim 32, wherein the amount of said analyte in said sample is quantitated.

39. A method of detecting the presence of an unknown analyte in a sample, comprising contacting said sample with the pore assembly of claim 25, detecting an electrical current through at least a first channel to determine a sample current signature, and comparing said sample current signature to a standard current signature of a known analyte, wherein a concurrence of said sample current signature and said standard current signature indicates the identity of said unknown analyte in said sample.

10 40. A method of detecting a change in the type or amount of a biological or chemical constituent in a sample, comprising:

- 15 (a) contacting said sample with the pore assembly of claim 25 at a first time point;
- (b) determining a first sample current signature by detection of an electrical current through at least a first channel;
- (c) contacting said sample with the pore assembly of claim 25 at a second time point;
- (d) determining a second sample current signature by detection of an electrical current through at least a first channel; and
- 20 (e) comparing said first sample current signature to said second sample current signature, wherein a difference between said first sample current signature and said second sample current signature is indicative of a change in the type or amount of a biological or chemical constituent in said sample.

30

41. The method of claim 40, wherein said first and second sample current signatures are detected through said at least a first channel in continuous flow mode.

42. A method of detecting a change in the physical environment of a sample, comprising:

- 5 (a) contacting said sample with the pore assembly of claim 25 at a first time point;
- 10 (b) determining a first sample current signature by detection of an electrical current through at least a first channel;
- (c) contacting said sample with the pore assembly of claim 25 at a second time point;
- 15 (d) determining a second sample current signature by detection of an electrical current through at least a first channel; and
- 20 (e) comparing said first sample current signature to said second sample current signature, wherein a difference between said first sample current signature and said second sample current signature is indicative of a change in the physical environment of said sample.

43. The method of claim 42, wherein said first and second sample current signatures are detected through said at least a first channel in continuous flow mode.